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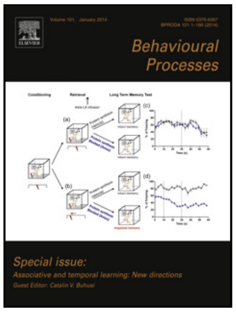

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Sniffing out the competition? Juvenile coral reef damselfishes use chemical cues to distinguish the presence of conspecific and heterospecific aggregations

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Highlights

- Species assemblages and depth preferences noted for 4 common coral reef damselfish.
- Juvenile fish subjected to pairwise olfactory choice tests.
- Chemical cues tested where conspecific and heterospecific.
- Three of four species demonstrated a preference toward conspecific cues.
- Responses toward heterospecific cues were neutral or negative.
- All olfactory cues derived from *P. moluccensis* elicited an avoidance response

ABSTRACT

Aquatic animals commonly rely on chemical cues to provide information regarding their surroundings. They can respond either by being attracted to (potential mates, preferred habitats) or avoiding (predators, competitors) the source of the stimuli. Coral reef fishes use chemical cues to detect habitats, avoid predators and recognise conspecifics. However, the extent to which chemical cues are used to detect and respond to potential competitors, has received little attention. Here we test olfactory preferences for conspecifics and heterospecifics in newly settled juvenile coral reef fishes. Juveniles of four common coral-associated damselfish species: *Dascyllus melanurus, Dascyllus reticulatus, Chrysiptera arnazae* and *Pomacentrus moluccensis,* were subjected to olfactory choice tests. Three of the four species (excluding *P. moluccensis*) demonstrated preferences for waterborne conspecifics odours. All species exhibited an avoidance towards heterospecific odours; this aversion was consistently greatest towards *P. moluccensis*. A neutral response toward heterospecifics was only evident in two instances 1) between the two congeneric *Dascyllus* species, with *D. melanurus* toward *D. reticulatus,* and 2) with *C. arnazae* toward *D. melanurus*. While it is already known that the presence of conspecifics plays a vital role in settlement site selection, we show here that the presence of heterospecifics may also be key in determining the spatial distributions of juveniles across areas of coral reef.

Key Words: *Olfaction, Chemosensory Cues, Group Assemblages, Conspecifics, Heterospecifics*

1. INTRODUCTION

Animals vary in the degree to which they are attracted to or avoid conspecifics or closely related species (e.g. Holmes & Sherman, 1983; Ward et al., 2007). Attraction is expected to occur if there are benefits to living in conspecific or heterospecific groups. The presence of other individuals can presumably indicate that a site is a suitable living area, particularly in terms of foraging opportunities and shelter from predators. Group living can also allow the individual more foraging or resting time (Seghers, 1981; Booth, 1992; Ben-Tzi et al. 2009). However, where intra and interspecific competition is intense, individuals may be expected to avoid potentially competing individuals (Jones 1987; Munday, 2001; Bostrom-Einarsson, 2013). Joining large aggregations may be costly, leading to refuge limitation and subsequent increasing the likelihood of density-dependent mortality (Shima, 2002). Furthermore, those deemed subordinates in these group assemblages are likely to suffer automatic fitness costs in terms of missed reproductive opportunities (Emlen, 1995). The ability to detect social situations and then decide whether or not to settle with a group of residents, therefore, is critical for survivorship and long-term fitness. (e.g. Lima, 1995; Robertson, 1996; Booth, 2002; Ben-Tzi et al., 2009; Ford & Swearer 2013). Thus, determining who is a friendly resident, and who is not, should be a highly selectable evolutionary trait.

To make the appropriate response, individuals rely on their sensory ability to identify one another (Ward et al., 2005, 2007) and through the detection of such cues, behavioural and physiological responses are mediated (Tierney *et al*, 2010). Chemical or olfactory cues are known to be of critical importance across a wide range of taxa (Wyatt, 2003), playing important roles in communication and habitat selection for numerous species (e.g. bats, lizards, lobsters, sticklebacks). This use of chemical cues is particularly important amongst aquatic organisms that have evolved in environments comprised of dissolved chemicals and many fish use chemical cues to detect and discriminate between conspecifics and heterospecifics (McLennan & Ryan, 2008). However, animals often live in diverse assemblages and the degree to which individuals can use olfactory stimuli to respond appropriately to conspecifics and a range of other potential competitors is not always known.

Numerous studies have attempted to elucidate the role of olfaction in fish (e.g. Bertmar & Toft, 1969; Solomon, 1973; Nevitt et al., 1994) and there is now a considerable body of research demonstrating the use of chemosensory cues in the recognition of conspecifics and heterospecifics within freshwater ecosystems. In 1994 Brown & Smith showed the ability of fathead minnows (*Pimephales promelas*) in distinguishing both familiar and unfamiliar shoalmates via chemosensory cues. Likewise, the Trinidadian guppy (*Poecilia reticulata*) have the capacity to recognise both familiar and unfamiliar individuals, furthermore females have the ability to distinguish between the odours of individual males (Watt & Shohet, 2003; Shohet & Watt, 2004). However, only relatively recent years have marine fish species played a focal role in these investigations. The degree to which olfaction plays a role in the life history choices of coral reef fishes is only recently starting to be recognised.

Coral reef fish assemblages are among the most diverse vertebrate assemblages, with numerous small species often found in small conspecific or heterospecific groups associated with discrete coral habitats where they shelter (Allen 1991; Fautin & Allen, 1997; De Brauwer et al., 2015). Several studies have documented the fitness benefits of living in groups (Sweatman; Booth & Wellington 1998; Lecchini & Nakemura, 2013). Reef fish species are also known to be strongly territorial, aggressively defending sites from both conspecifics and closely related species (Robertson, 1984,1996; Ebersole, 1985; Bay et al. 2001; Ahmadia et al. 2012). Juvenile reef fish join resident reef populations following a brief pelagic larval stage, and must make rapid decisions on which species they will benefit from being attracted to and which species they should avoid. However, their ability to discriminate among multiple species at this critical life history stage has received little attention.

Reef fish species hatch with a well-developed olfactory system (Leis et al*.,* 2011; Dixson et al., 2012) and they are adept at using this to respond to a range of olfactory stimuli. Reef fish settlers are capable of distinguishing between water of oceanic origin and that derived from areas of reef (Atema et al., 2002; Gerlach et al., 2007), of differentiating amongst individual reef zones, or microhabitats (Dixson et al., 2008; Lecchini & Nakamura, 2013), and in locating the presence of other individuals (e.g. Sweatman, 1985, Lecchini et al., 2005a, 2005b, 2007), particularly predators (McCormick & Manassa, 2008; Lönnstedt &

McCormick, 2011). The presence of conspecifics has long been regarded as important in settlement site selection (e.g. Sweatman, 1983, 1985, 1988; Lecchini & Nakamura, 2013), with numerous individuals garnering information regarding their surroundings through the presence of conspecific individuals, rather than the habitat itself (Stamps & Krishnan, 2005). To date however, the majority of sensory research on reef fish has focused on environmental cues derived from solely from different habitat types. The role of olfactory cues in conspecific and heterospecific attraction and avoidance has received much less attention.

In this study we examine the role that olfactory cues play in conspecific and heterospecific recognition, including attraction and avoidance, in a guild of coral reef dwelling damselfish species: *Dascyllus melanurus, D. reticulatus*, *Chrysiptera arnazae* and *Pomacentrus moluccensis*. All species exhibit similar feeding behaviours, life spans and home ranges, and demonstrate persistent (if not obligate) relationships with branching corals (Shpigel, 1982; Forrester, 1990). Additionally, all four species use the same types of branching coral colonies (Kuiter & Tonozuka, 2001; Bonin et al., 2009). However, they vary in the degree to which they form conspecific and heterospecific groups. We begin by quantifying the frequency with which individuals of each species occurred in group assemblages with both conspecifics and/or the three heterospecific species, including an analysis in the overlap in their depth distributions. We then undertake pair-wise olfactory choice experiments to determine whether responses to chemical cues can explain the degree of co-habitation or apparent avoidance. Four specific hypotheses were tested: a) species that commonly occur in conspecific groups will respond positively to the smell of their own species, likewise, b) species that are commonly found in heterospecific group assemblages will be positively attracted to the smell of any heterospecific species that they frequently live with, c) for those species that do not occur in conspecific groups, neutral or negative olfactory responses will be apparent, and lastly d) species that do not commonly form heterospecific groups will elicit a neutral or negative response toward the smell of other species. Ethical authorisation for this study was covered under James Cook University approval number A1837.

2. MATERIALS AND METHODS

2.1 Study Location and Species

The study was carried out at Kimbe Bay, Papua New Guinea (5°12.530 S, 150°22.801 E), between April 2012 and October 2013 at Mahonia Na Dari Research and Conservation Centre. The focal species for this study were four species of damselfish (Pomacentridae): *Dascyllus melanurus*, *Dascyllus reticulatus*, *Chrysiptera arnazae* (formerly *C. parasema*) and *Pomacentrus moluccensis.* All four species recruit to live hard coral colonies, and particularly branching *Acropora*, *Pocillopora* and *Seriatopora* colonies (Ault & Johnson 1998; Brunton & Booth 2003; Bonin et al., 2009).

2.2 Conspecific and heterospecific groups

To evaluate whether olfactory responses towards social cues contributes to the dispersion of recruit fish on reefs, we first explored the natural, in-situ dispersion of the focal species. For each of the four damselfish species we determined how much overlap there was in their use of reef space, both among conspecifics and heterospecifics. Natural distributions were evaluated by surveying the leeward and fore-reef slopes of 14 inshore reefs in Kimbe Bay. A 150m long x10m wide belt transect was carried out on each of the reef sites. The 10m width of the transect ensured that all depths reefs were surveyed, with very little overlap between transects, down to 15m. Records were then taken for each distinct aggregation of the focal species within these transects. Care was taken to ensure no aggregation was surveyed twice. Individuals or small groups moving between colonies were excluded due to difficulties in determining distinct aggregations.

For each aggregation, we recorded the specific depth, abundance, and the taxonomic identity of all focal damselfish present. The approximate size of each focal species was also noted in order to distinguish recruit fish distributions from adults. Size estimations fell into one of three categories: New Recruits (NR) (<2.5cm SL), Juveniles (J) (2.5 - 5cm SL) or Adults (A) (>5cm SL). Any heterospecific species present within the group assemblage were also recorded. In order to verify our size estimations, samples were collected for each size category (for each species) and measured with callipers prior to conducting the transect surveys.

2.3 Olfactory discrimination trials

Newly recruited juveniles (<2.5cm SL) of each fish species were collected from small coral heads (approx. 10x10cm in surface area) in 2-15m depth using hand nets and clove oil solution (1:2:3, clove oil: low grade ethanol (94%): seawater (Munday & Wilson, 1997)). Following their capture fish were given a minimum 2-hour recovery period, whilst housed in aquaria with other conspecifics. All fish demonstrated active swimming behaviour in the holding aquaria, indicative of recovery from the anaesthetic. Fish that were housed in aquaria for periods exceeding 12 hours were fed live *Artemia* nauplii following exposure to the trials. Newly recruited juveniles were used instead of 'naïve' larvae caught in light traps, as this provided an adequate sample size for the experimental tests. Although previous studies have shown evidence of rapid ontogenetic change in morphology and behaviour at and / or soon after the point of settlement (Leis & Yerman 2012), the majority of studies, to date, that have compared larval versus juvenile responses to olfactory stimuli have demonstrated little or no variation in choices, regardless of ontogenetic phase (e.g. Öhman et al., 1998; Dixon et al., 2008; Roux & Lecchini, 2015). Thus inferring settlement choices from the choices of newly recruited juveniles is possible. Additionally, the low recruitment seasonality in equatorial reef fish populations leads to unproductive light-trap sampling effort at this locality (Srinivasan & Jones, 2006), such that newly settled recruits were the most feasible sampling unit to use.

2.4 Apparatus and trial design

The responses of juvenile *D. melanurus, D. reticulatus, C. arnazae* and *P. moluccensis* to olfactory cues were tested using a two-channel choice-flume (13cm x 4cm), developed by Atema et al. (2002). The apparatus allows for pairwise choice experiments to be carried out, in which individual fish can move freely between water flowing from two different sources. Water from two alternate sources was gravity fed into the choice-flume, which was partitioned along half its length. Recruits were released in the downstream end of the flume, where the exploration of both water sources was possible, allowing for the selection of a preferred source. Water flow was maintained at a constant speed of 100ml min^{-1} throughout all trials and dye tests were conducted before each trial to ensure that laminar flows were apparent

For each trial, a single recruit was placed in the downstream end of the flume and left to acclimate for two minutes. During this period the fish was able to swim throughout the chamber. At the end of the acclimation period, the position of the fish within the chamber was recorded at five-second intervals for the duration of two minutes. This was followed by a one-minute rest period, during which the water sources were altered, providing a control for potential chamber side preferences. Following this alteration the entire test, including the acclimation period was repeated. Any fish that did not swim throughout the chamber during the acclimation period were removed from the trials, as it was not deemed to have assessed the choices available. Of the 357 fish acclimated 39 were removed prior to trials being conducted. These numbers were 9, 7, 11 and 12 for *D. melanurus*, *D. reticulatus*, *C. arnazae* and *P. moluccensis* respectively.

The experimental design consisted of juveniles given the choice between either a water source that had been treated with a specific chemical cue (conspecific or heterospecific) and a water source that had not been treated with any chemical cues (the control). Each species was tested, both for conspecific responses and responses to the other three species. Reef water collected from the same reef as the juvenile fishes was used as a base 'control' water source throughout the trails and collected daily. Chemical discrimination trials were run on 15-20 individual fish of each species per treatment. No fish was used more than once.

2.5 Treatment preparation

Conspecific and heterospecific treatment water was prepared by holding 10-15 juveniles and/or new recruits in 50 l of reef water, with an air source, for a period of 24 h. Fish were captured using a combination of low dose clove oil (one part clove oil, two parts low-grade ethanol (94%) and three parts seawater), barrier nets and hand nets. Heterospecific water treatments consisted of a singular species. Several water preparations, involving different species were used when testing for preference or avoidance reactions throughout the trials.

2.5 Statistical analysis

To compare differences in fish dispersion among depths and thus assess whether the 4 species shared the same niche space, a one way Analysis of Variance (ANOVA) was conducted on mean depth use. This was followed with Tukey's HSD test to discern group membership. Assumptions of normality and homogeneity were verified with QQ plots, Brown-Forsythe's test and residual plots. The frequency of social associations among conspecifics and heterospecifics was compared graphically.

Experimental results were analysed with Kolmogorov-Smirnov (K-S) tests. We used these to compare the total proportion of time that individual recruits spent in different streams of water (sensu: Munday et al., 2009; Dixson et al., 2010). The time spent in treatment water was recorded for each individual by summing the number of 5-second intervals a fish spent in the treatment water over the total 4-minute period (max count: 48). The median was calculated from the 20 replicate trials for each comparison. The null hypothesis was that the presence of chemical cues would provoke a limited response, thus a uniform distribution would occur between the two water sources. Where observed and expected distributions differed significantly a preference for a particular water source could be stated. Thus, prior to statistical analysis it was hypothesized that no significant differences would arise, for any species, between 1) the presence of conspecific cues versus control water (untreated reef water), nor 2) the presence of heterospecific cues and control water (untreated reef water).

3. RESULTS

3.1 Prevalence of conspecific and heterospecific groups

In total 879 groups, with approximately 9,800 individuals, of the focal damselfish species were surveyed on Kimbe Bay reefs. All fish were found in aggregations ranging in size from 3-200 individuals (Table 1). No new recruits (<2.5cm) were ever observed on their own, but were always found in groups of at least 3 conspecifics. Conspecifics were frequently found with other recruits and juveniles (2.5-5cm). No recruit fish were ever seen in groups comprised of adults (>5cm) alone.

Differences in species depth associations were apparent (Fig. 1) (ANOVA: ($F_{(3,1581)} = 267.7$, $p = 0.0001$), $R^2 = 0.3368$; Tukey's HSD $p<0.0001$ in all instances, with the exception of *D. melanurus* vs. *P. moluccensis* where $p<0.99$). *P. moluccensis* predominantly occurred in the shallow waters along the reef crest (2-3m), while, *C. arnazae* primarily inhabited the 10m areas. *D. melanurus* and *D. reticulatus* showed used similar associations to each other, most frequently occupying the shallow-mid depth zones. QQ plot results showed normal distributions and homogeneity throughout.

Group sizes differed among the species. *Pomacentrus moluccensis* was found in the smallest groups, typically with 3 fish and with a maximum of 40 individuals. The mean group size of *P. moluccensis* was 50% smaller than aggregations of the other 3 species (Table 1). In contrast, *C. arnazae* exhibited the largest conspecific groups, with a maximum of 200 individuals being observed (although similarly the most frequent group size contained 3 fish). *D. melanurus* and *D. reticulatus* demonstrated comparable mid-range group sizes with the largest observed groups comprising of 75 and 50 individuals respectively. However, the most frequent group sizes, for three of the species (*D. melanurus, D. reticulat*us and *C. arnazae*), were larger when conspecific adults were absent (Table 1.). The amount of space shared with heterospecifics differed substantially between species, and in some instances, was fairly limited (Fig. 2). *D. melanurus* and *D. reticulatus* were most commonly seen sharing habitat resources, with 25% of all *D. melanurus* observations incurring *D. reticulatus* sightings and conversely 20% of *D. reticulatus* observations were with *D. melanurus* (Table 1). However, neither *C. arnazae* nor *P. moluccensis* associated regularly with heterospecifics. The percentage occurrence of co-habitation was ≤5% in each case (Table 1, Fig. 2).

3.2 Conspecific odour cues

The three most naturally gregarious species had strong and significant preferences towards conspecific odours (Fig. 3). *D. melanurus* recruits spent two thirds of their time in conspecific treated water rather than the untreated water (KS = 0.533, $p<0.005$). Likewise, significant preferences for chemical cues from conspecifics were detected for *D. reticulatus* (KS = 0.55, $p<0.0047$) and *C. arnazae* (KS = 0.667, $p<0.0001$), which spent an average of 61% and 65% of their time in conspecific-soaked water, respectively. In contrast to these species, *P. moluccensis,* showed a neutral response toward conspecifics. It spent just over half the trial time in conspecific seeded water rather than untreated water (56%, KS = 0.3, $p<0.32$).

3.3 Heterospecifics odour cues

D. melanurus exhibited a range of olfactory responses to heterospecifics (Fig. 4). A neutral response was apparent toward the presence of *D. reticulatus* (53%, KS = 0.23, p = 0.59). Strong aversions to water seeded with chemical cues from *P. moluccensis* (57%) and *C. arnazae* (69%) occurred (KS = 0.45, p<0.05 and KS = 0.75, p<0.0001 respectively).

D. reticulatus demonstrated a significant avoidance of all three heterospecific test species (Fig. 4). However, avoidance was 10% stronger towards *C. arnazae* and *P. moluccensis* than towards *D. melanurus* (69%, KS = 0. 75 p<0.0001; 67%, KS = 0. 9, p<0.001; 57%, KS = 0.65, p<0.0004 respectively).

C. arnazae demonstrated a neutral response toward *D. melanurus* (59%, KS: 0.4, p<0.0815) but showed notable avoidances toward the remaining two heterospecific test species used; *P. moluccensis* (76%, KS: 0.9, p<0.0001 and *D. reticulatus* (68%, KS: 0.6, p<0.015)(Fig. 4). This evasion was greatest towards *P. moluccensis*.

P. moluccensis exhibited very strong aversions toward all three heterospecific test species (*D. melanurus, D. reticulatus* and *C. arnazae*). The percentage time spent in water without heterospecific associated chemical cues exceeded 70% in all three cases (70%, 73% and 72% respectively) (Fig. 4). This result was statistically significant (in all three instances KS = 0.85, p≤0.0001).

4. DISCUSSION

The observed attraction and avoidance of water-borne chemical cues within and among the four damselfish species was consistent with the patterns of co-occurrence in the field. We show that individuals of the focal species are capable of species-specific responses in these diverse reef fish communities. Recruits of three gregarious and coral-associated damselfish species demonstrated strong olfactory preferences toward the presence of conspecifics when tested against reef water containing no specific olfactory cues. A fourth species, which is less gregarious at settlement, showed neutral responses. In contrast, the detection of chemical cues emitted from heterospecifics in most instances elicited a significant avoidance. These avoidances were strongest where either, species occupied distinctly different habitat areas, or when the formation of heterospecific groups was uncommon. Attraction towards the smell of heterospecific water did not occur. There were only two instances in which neutral responses were exhibited toward the scent of a heterospecific species. This occurred between the two sister species that frequently share the same coral refugia *Dascyllus melanurus* and *D. reticulatus* and also by *C. arnazae* in response to chemical cues derived from *D. melanurus*. Overall, the olfactory responses demonstrated by all species, for the most part, mirrored the spatial dispersion of conspecific and heterospecific individuals seen on the reef. We therefore conclude that the conspecific and heterospecific olfactory cues detected by reef fish during their settlement phase contribute to their interspecific spatial distribution and dispersion patterns.

This study is one of the first to demonstrate the potential role of olfactory cues in conspecific attraction and heterospecific spacing on coral reefs. The effects of social attraction, and the resulting resource preferences are well known in many animal systems (e.g. seabirds (Hutto, 1985), lobsters (Briones-Fourźan et al., 2008) and in fresh water fishes such as sword-tails (McLennan & Ryan, 1997) and sticklebacks (Ward et al., 2003, 2005, 2007)). Social preferences are also known to be strong during reef fish settlement phases (e.g. Sweatman, 1985, 1988; Sweatman & St John 1990; Lecchini & Nakemura, 2013) with the presence of conspecifics favouring outcomes of survival, competition, foraging and access to shelter (Beukers & Jones, 1997; Booth, 2002; Ben-Tzi et al., 2009). Whilst many of these previous studies have demonstrated the importance of other individuals at times of recruitment (e.g. Booth 1995), a number of these studies demonstrate this recognition through the use of multiple sensory cues (e.g. Lecchini et al., 2005b; Wright et al., 2005; Huijibers et al., 2012).

The detection of conspecifics solely via chemical cues and conspecific attraction noted here supports a number of previous studies conducted on gregarious reef fish species (e.g. Coppock et al., 2013; Lecchini & Nakamura, 2013). Whereby the presence of conspecifics is seen a critical in habitat selection, thus informing subsequent patterns of dispersion and abundance. The neutral response elicited by *P. moluccensis* indicates that for some less gregarious damselfish species, the presence of conspecifics is likely to be secondary to other key determinants in spatial distribution such as habitat type, habitat quality or the locality of potential competitors. The role of heterospecific chemical cues, with regard to detecting potential competitors, has received much less attention. This study demonstrates that the ability of damselfish species to distinguish heterospecifics extends beyond merely detecting the presence of predators and encompasses a much finer degree of identification than previously anticipated, extending as far as the identification of other species within the same guild, consequently having the ability to identify competitors.

For those species that often shared space on the reef (*Dascyllus* species), responses to heterospecific cues were mixed. *D. melanurus* exhibited a neutral response to chemical cues emitted by *D. reticulatus.* This response was perhaps unsurprising given that the results from the in situ distributions showed that *D. melanurus* occurred twice as often with its sister species *D. reticulatus*, than with either the *Pomacentrus* or *Chrysiptera* species. This response, however, was not mirrored by *D. reticulatus*. Despite *D. reticulatus* regularly inhabiting same coral colonies as *D. melanurus*, the olfactory trial results indicate that *D. reticulatus* appear to avoid the former, upon detection, rather than chose it. Asymmetric competition has previously been demonstrated between *D. flavicaudus* and *D. aranurus* a congeneric and morphologically very similar sister species to *D. melanurus* (Schmitt & Holbrook, 1999). Whilst both species were seen to co-habit the same coral colonies, the subsequent population growth rates for *D. aranurus* were dramatically reduced in the presence of *D. flavicaudus.* It is entirely possible that a similar relationship occurs between *D. melanurus* and *D. reticulatus*. Thus while the presence of *D reticulatus*, for *D. melanurus*, may indicate the occurrence of suitable group assemblages (in a similar manner to conspecifics), for *D. reticulatus* individuals detecting the presence of it's sister species is not beneficial. Here, where possible, it must be advantageous to avoid co-habitation thus minimising potential implications toward their future survival and fitness, hence the aversion witnessed.

With respect to *C. arnazae,* the reason behind the neutral response seen toward heterospecific cues derived from *D. melanurus* is unclear*. Dascyllus melanurus* is known to be the competitively dominant species in its relationship with *C. arnazae* (Bonin et al 2009*;* Bostrom-Einarsson et al. 2014) and as such a significant avoidance might have been expected. However, *C. arnazae* and *D. melanurus* rarely appear to share reef space, inhabiting for the most part distinctly different depths, thus presenting little threat to one another in terms of competition for resources. In this instance, perhaps, the presence of suitable resources might override the presence of potential competition.

In all other instances chemical cues derived from heterospecific species acted as a deterrent. This heterospecific avoidance was evident both amongst those species known to co-exist and between those that had markedly difference depth ranges with minimal co-habitation. Much of the avoidance observed could be expected if interspecific competition was prevalent among species in this guild. Competitively dominant species are known to increase mortality rates of subordinate species or limit the abundance of subordinate species by restricting access to mutually preferred habitats (Robertson, 1996; Munday 2001; Bostrom-Einarsson et al. 2014, Bonin et al, 2015). All species, including *C. arnazae* demonstrated a strong avoidance of cues derived from *P. moluccensis.* This response was notably greater than those seen in any of the other heterospecific trials. *P. moluccens*is were primarily noted in small conspecific groups, within much shallower depth range than the other species in question. Their presence in heterospecific groups was rare. A factor that was accurately mirrored by the heterospecific olfactory responses.

Contrary to our initial hypothesis, the strongest aversions to heterospecific odour cues were displayed between the two species (*P. moluccensis* and *C. arnazae*) that occupied different depth ranges, exhibited slightly different habitat preferences and were rarely noted in heterospecific groups. *P. moluccensis* in particular are known to be aggressive competitors for their space on the reef, pushing out heterospecific species (Bonin et al., 2009; Bostrom-Einarsson et al., 2013, 2014). Presumably, the detection of the heterospecific cues, in these instances, indicates either the presence of an aggressive competitor, or the existence unsuitable living conditions.

Limitations to this study must be acknowledged. Previous investigations have shown evidence of rapid ontogenetic change in morphology and behaviour at and / or soon after the point of settlement (Leis & Yerman 2012). Thus, preferences for coral species may differ depending on whether juveniles are tested before or after settlement (Danilowicz, 1996). Since all our focal individuals had already settled, patterns of choice may not necessarily reflect choices that were made at settlement. However, almost all studies that have compared settlement choices in pre-settlement larvae and post-settlement juveniles have found identical patterns, regardless of ontogenetic phase (e.g. Öhman et al., 1998; Dixon et al., 2008; Roux & Lecchini, 2015). For these species, therefore, it may be possible to infer settlement choices in pre-settlement larvae through olfactory trials carried out on post-settlement juveniles, as performed here.

In conclusion, this study presents evidence that olfactory-based species specific recognition is an important mode of communication in damselfishes and can play a role in determining the local dispersion of reef fish species. Our results reiterate the attractive importance of conspecific chemical cues at times of settlement. We also reveal the importance of heterospecific olfactory cues in shaping species distribution across coral reefs, particularly for gregarious species. Future research now needs to expand upon the current study to establish the extent to which the importance of heterospecific cues might alter amongst more territorial damselfishes and whether this is a reef wide phenomenon.

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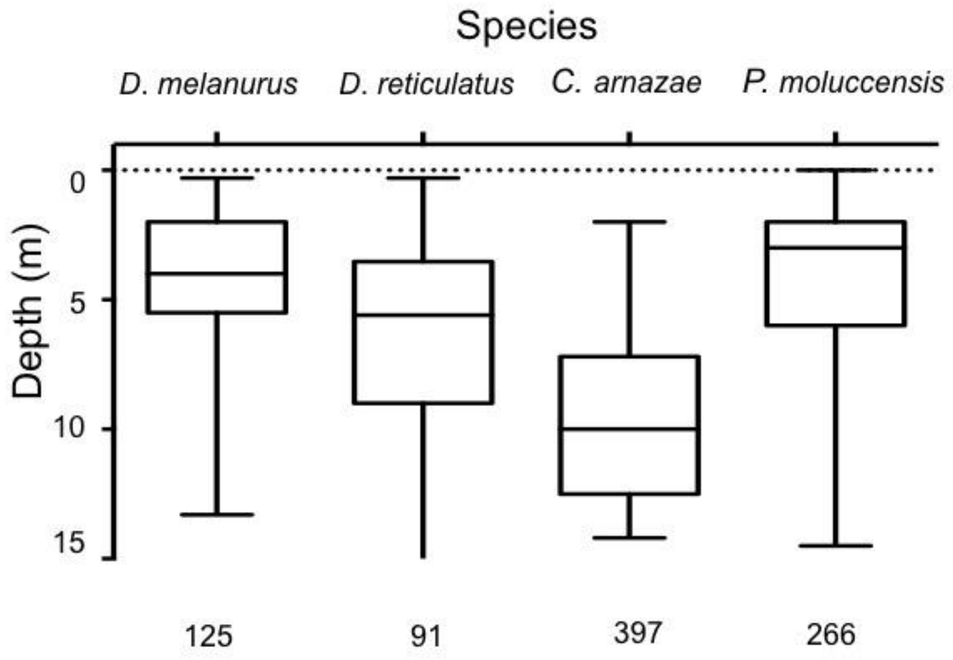

Figure 1. Depth distributions of the four damselfish species; *Dascyllus melanurus*, *D. reticulatus*, *Chrysiptera arnazae* and *Pomacentrus moluccensis,* including the mean depth (horizontal line), SE (vertical rectangle) and total depth range (vertical line). Surveys were conducted down to a maximum depth of 15m. The number of groups observed per species, are denoted beneath the box plots.

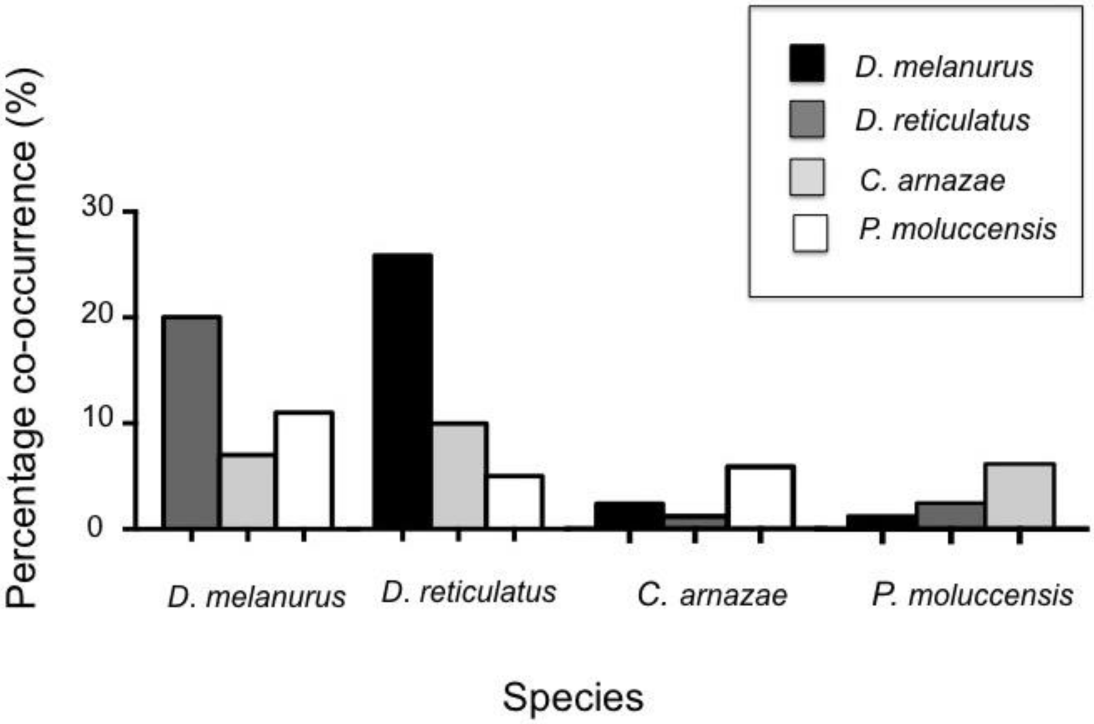

Figure 2. Social situations: the dispersion of four common damselfish species over live coral colonies. Percentage of instances in which test species were seen co-habiting. Columns show the proportion of instances (%) in which species occupied the same refugia.

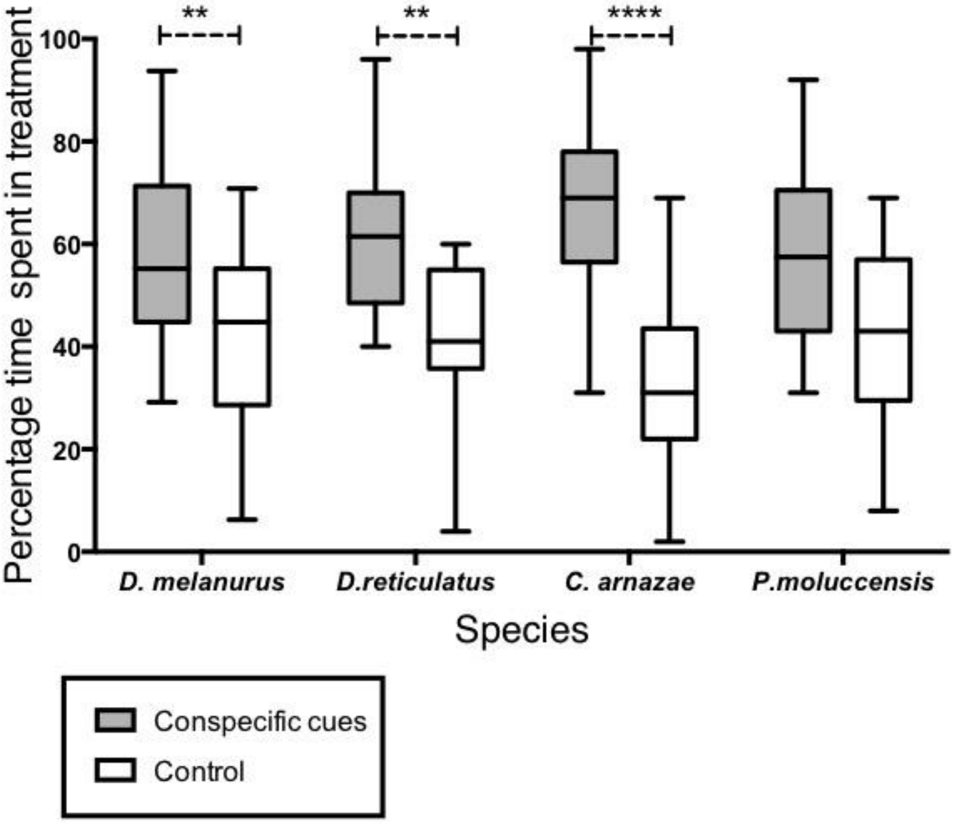

Figure 3. Response of *Dascyllus melanurus, Dascyllus reticulatus, Chrysiptera arnazae* and *Pomacentrus moluccensis* to olfactory cues from conspecifics. Columns show the mean percentage time (± SE) that recruits spent in cue treated water (grey bars) versus water containing no cue (white bars). Significant differences are signified by the presence of asterisks, **p≤ 0.005,: **** p≤ 0.0001

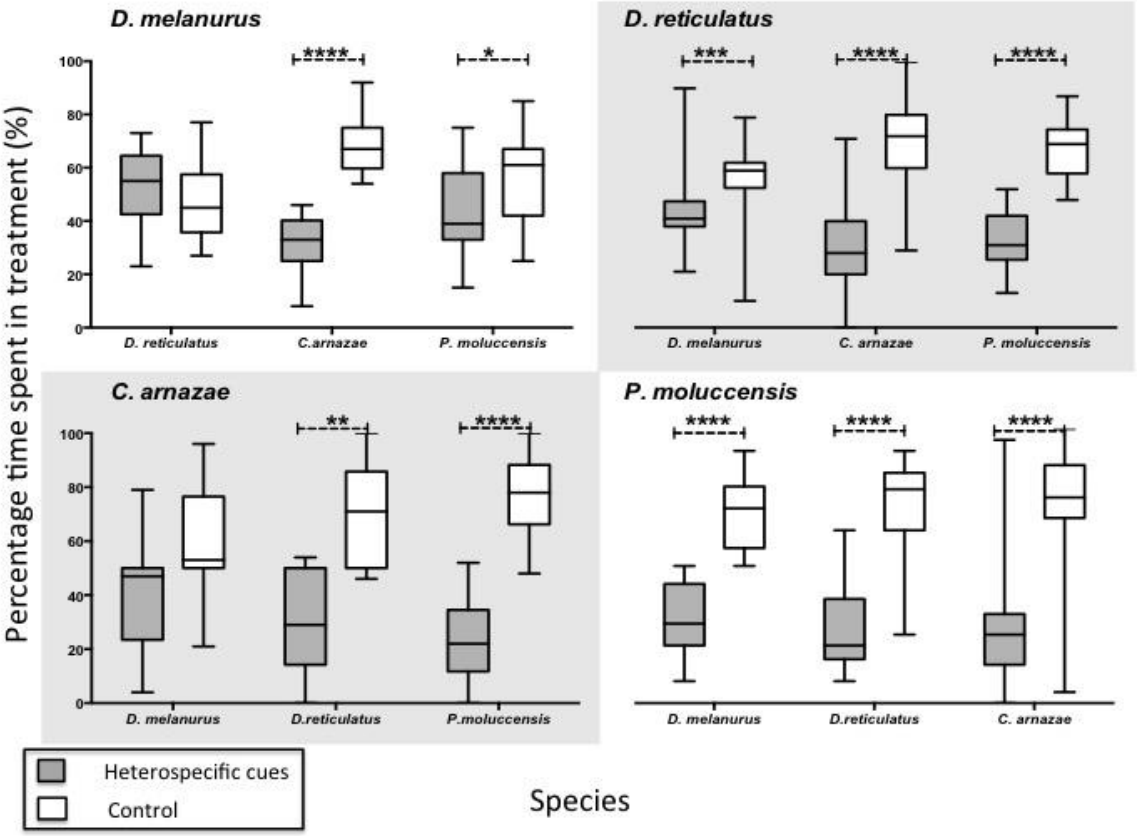

Figure 4. Response of *Dascyllus melanurus, D. reticulatus, Chrysiptera arnazae* and *Pomacentrus moluccensis* to olfactory cues from heterospecific species. Columns show the mean percentage time (± SE) that recruits spent in cue treated water (grey bars) versus water containing no cue (white bars). Species used as heterospecific cues are denoted along the x-axis. Significant differences are signified by the presence of asterisks: *p≤0,05, **p≤ 0.01, ***p≤0.001, ****p≤0.0001

Table 1. The dispersion of four common damselfish species over live coral colonies on Kimbe Bay (PNG) reefs. Data are the number of observations (n), average group size (mean), mode group size (when adults were present and absent), and range., The proportion of group assemblages that consisted purely of conspecifics (%), the proportion of group assemblages with any heterospecifics present, and the proportion of instances in which species were found with focal Pomacentrid species (*Dascyllus. melanurus, D. reticulatus, Chrysiptera arnazae* or *Pomacentrus moluccensis*) are also shown.

Species	No. of Group Assemblages (n)	Range (Individuals)	Mean Group Size	Mean Group Size (NR & J)	Mode Group Size	Mode Group Size (NR & J)	Conspecifics Only (%)	Heterospecifics (Any) (%)	Heterospecifics (Pomacentrid) (%)
Dascyllus melanurus	125	3-75	12	12	3	5	25.6	74.4	44
Dascyllus reticulatus	91	3-50	11	11	3	5	53.8	46.2	35
Chrysiptera arnazae	397	3-200	12	15	3	6	45.1	54.9	8
Pomacentrus moluccensis	226	3-40	6	6	3	3	42.5	57.5	8